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Predation on walleye pollock (*Theragra chalcogramma*) eggs and yolk-sac larvae by pelagic crustacean invertebrates in the western Gulf of Alaska

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ABSTRACT

Immunological detection of yolk protein was used to assess predation by pelagic amphipods (gammarid and hyperiid), mysids, and euphausiids on eggs and yolk-sac larvae of walleye pollock *Theragra chalcogramma* during 1988 and 1989. Consumption estimates were made on the basis of frequency of positive immunoassays, assay detection times (gut clearance time), predator abundance, and spatial overlap of predators and prey. From our results gammarid amphipods and euphausiids were important predators on eggs and yolk-sac larvae, respectively. Gammarid amphipods alone consumed about 14% of the standing stock of pollock eggs in 1989. These results were compared with those from clearance rate experiments of predators feeding on pollock eggs in 300-l bags. In general, clearance rate estimates of egg consumption were lower than those determined from gut contents.

Key words: walleye pollock, *Theragra chalcogramma*, predation, fish eggs, immunoassay

INTRODUCTION

Predation is believed to be one of the major sources of mortality for marine fish eggs and larvae (Hunter, 1984; Bailey and Houde, 1989, and references therein). However, predation in the field, including identification of the major predators and how many prey they are eating, is poorly understood owing to difficulties of assessment.

Several methods are used to study invertebrate predation upon early stages of marine fishes, each with advantages and drawbacks. Laboratory-derived clearance rates of predators feeding on fish eggs and larvae have been applied to field estimates of predator abundances (Bailey and Yen, 1983; Monteleone and Duguay, 1988). This method has numerous problems; for instance, small-scale spatial overlap of prey and predators is not easily replicated in the laboratory, and it is difficult to extrapolate results under laboratory conditions to those in the sea. Predation also can be estimated from the difference between total and nutrition-related mortality (Hewitt et al., 1985; Leak and Houde, 1987); drawbacks to this method are that predators remain unidentified and both empirical estimates may be relatively imprecise, resulting in a low degree of confidence in predation estimates.

Gut contents of potential predators can be examined either visually or serologically. Difficulties with these methods include variable digestion rates, gut voiding, and feeding during net capture. Visual examination of guts has been particularly useful for studying fish predation on eggs (Hunter and Kimbrell, 1980; Brodeur et al., 1991), but larvae are believed to be digested very rapidly. This method has also been useful for estimating predation of gelatinous zooplankton on eggs and larvae (Moller, 1984; Purcell, 1989; Purcell and Grover, 1990). Serological techniques are most useful for marine crustacean predators that macerate their prey, which renders direct visual identification impossible (Feller et al., 1979). Theilacker et al. (1986) and Theilacker (1988) have used serological methods to study predation by euphausiids on northern anchovy *Engraulis mordax* eggs and yolk-sac larvae.

We studied invertebrate predation on walleye pollock (*Theragra chalcogramma*) eggs and yolk-sac larvae from Shelikof Strait, the major spawing area in the western Gulf of Alaska (Kendall and Picquelle, 1990). Peak spawning occurs during a two-week period in early April. Eggs are located near the bottom (150–250 m) in a concentrated patch (Kendall and Kim,

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1989) and hatch after about 14 days at a water temperature of 5–6°C. Yolk-sac larvae (3–4 mm) move toward the surface and are generally distributed in the upper 150 m; they are still quite concentrated and located downstream from the eggs (Incze et al., 1989).

Mortality of walleye pollock eggs and early-stage larvae is high and variable. Daily egg mortality has been estimated at 10–30% (Kim and Gunderson, 1989). Estimates of daily larval mortality range from 6% to 15% (Incze et al., 1989; Yoklavich and Bailey, 1990). It has been proposed, although the evidence is scarce, that predation is the major source of walleye pollock egg (Kim and Gunderson, 1989) and larval mortality (Walline, 1983).

The objectives of the present study were (1) to identify invertebrate predators of walleye pollock eggs and yolk-sac larvae using serological techniques, (2) to estimate predation rates in the field, and (3) to compare predation rates in the field to those determined from clearance rates in 300-l bags.

MATERIALS AND METHODS

Field methods

The area of the main walleye pollock spawning aggregation in Shelikof Strait was initially sampled in a 18.5 × 18.5 km grid to define the distribution of pollock eggs and larvae during ichthyoplankton surveys in 1988 and 1989. Following these initial presurveys, plankton collections were made in areas of high egg and yolk-sac larval densities. Cruises were conducted aboard the NOAA ship *Miller Freeman*. Cruises from April 1 to 13, 1988 and from April 5 to 16, 1989, were termed "egg cruises," since eggs were predominant in catches. A cruise from 16 April to 2 May 1988 was termed the "yolk-sac cruise" even though eggs were also present, since hatching was occurring and sampling was directed to capture predators feeding on yolked larvae.

A 1-m^2 mechanically operated Tucker trawl with 505-μm mesh nets was deployed to sample predators at specific depth strata where the highest concentrations of eggs and yolk-sac larvae were expected to occur (Kendall and Kim, 1989; A. Kendall, NMFS, Seattle, personal communication). For egg predators the net was towed obliquely from 250 to 150 m. For yolk-sac larvae the net was towed obliquely from 150 m to the surface. Depth of sampling was monitored with an electronic bathykymograph. Volume of water sampled was estimated with a flowmeter in each net. To examine diel differences in predation, we tried to distribute the sampling effort equally between day and night.

Larger predators also were sampled during the yolk-sac cruise in 1988 using a 5-m^2 Methot frame trawl (Methot, 1986) equipped with a 2 × 3 mm oval mesh net. Oblique tows were made from 150 m to the surface.

Feeding on eggs and larvae in the codend of the nets (Hirota, 1984; Nicol, 1984) is a potential problem in sampling. A 1.5-mm mesh codend was used on all nets to allow pollock eggs and larvae to pass through the codend while predators were retained.

The contents of the codend were emptied into a sorting tray containing a slurry of iced seawater, and most of the large crustacean predators were removed quickly (<10 minutes), placed in microcentrifuge tubes, and stored at −80°C. The remaining sample was preserved in 5% buffered formalin in seawater. All specimens ≥5 mm (maximum dimension) in the crustacean groups Euphausiacea, Mysidacea, and Amphipoda (Hyperiidea and Gammaridea) were later identified to species and enumerated. Abundance was standardized to number per 10 m^2.

Because many predators make extensive vertical migrations extending outside the depth of our sampling, we adjusted predator abundance on the basis of the percentage of the total predator abundance that overlapped with egg or larval vertical distributions during the diel period. We approximated the vertical distribution of the dominant species in the four invertebrate groups and walleye pollock eggs and 3–4 mm larvae, presumed to be yolk-sac stage, using abundance data from four (two day and two night) MOCNESS tows (Wiebe et al., 1976), which were collected over eight depth strata from the surface to 250 m on April 21 and 24, 1988. Mesh size was 153 μm.

Laboratory methods

For immunoassays, individual frozen specimens were placed on a microscope slide and allowed to thaw briefly. Digestive tracts were dissected from each specimen and placed into Protein Extraction Buffer (PEB; 10 mM Tris-HCl, 1 mM EDTA, pH 7.4). Gut fullness was visually estimated and recorded. Guts were transferred into 20 μl of chilled PEB and homogenized with a Duall disposable tissue grinder (1.5-ml volume; Kontes of California, Hayward, CA). Ten microliters of homogenate were spotted onto 0.45-μm nitrocellulose paper in a microfiltration apparatus and allowed to drain. The blots were kept at 4°C until the membrane appeared to be dry, usually 12–18 hours.

The nitrocellulose membrane was removed from the filtration apparatus and blocked in Blotto-wash (5% nonfat dried milk, 0.05% Tween-20, 0.01% antifoam A, phosphate buffered saline, pH 7.4; modifed from

Theilacker et al., 1986) for up to 24 hours. The remaining protocol for washes and rinses is described by Theilacker et al. (1986) with the following modifications. Ten percent normal goat serum was used in both the conjugate antibody preblock and the conjugate antibody incubation. Because nonspecific binding of euphausiid gut contents appeared to occur with the secondary antibody, two blots were run simultaneously; one was incubated with both the primary and the secondary antibody, and the second was incubated with the secondary antibody only. The primary antibody was a polyclonal rabbit-anti-pollock egg-yolk protein IGG fraction produced in New Zealand rabbits (Merati et al., unpublished manuscript). The primary antibody used in the immunoblots was diluted 1 : 5000 in Blotto-wash. The secondary antibody was a 1 : 3000 dilution of goat-anti-rabbit alkaline phosphatase (Biorad Laboratories, Richmond, CA). Invertebrate specimens whose guts were allowed to clear for 24 hours and walleye pollock eggs and yolk-sac larvae were used as negative and positive controls, respectively. The reaction was visualized by color development with NBT (*p*-nitro-blue tetrazolium chloride) and BCIP (5-bromo-4-chloro-3 indyl phosphate *p*-toluidine salt).

Consumption estimates

The number of walleye pollock eggs or yolk-sac larvae consumed per 10 m^2 per day (C) by each predator species (j) was calculated by

$$C_j = \frac{A_j \cdot P_j \cdot N_j \cdot V_j}{D_j},$$

where

A = predator abundance per 10 m^2,

P = proportion of invertebrate guts testing positive for antigen,

N = number of eggs or yolk-sac larvae per gut (assumed = 1),

V = correction factor for incomplete vertical overlap (as described above),

D = detection time of yolk protein in guts (d).

Detection time (D) of yolk protein was estimated by observing predators placed in jars with walleye pollock yolk-sac larvae. Predators that fed successfully were placed in jars containing either filtered seawater or algae at 6°C (average water temperature in the field) and were sacrificed at hourly intervals. These predators were frozen in liquid nitrogen and assayed for egg proteins.

The vertical overlap correction factor (V) was estimated from the percentage of predators found at each depth stratum (i) during the day (P_{di}) and night (P_{ni}), adjusted to the same depth stratum (A_i) where eggs and larvae are found by the following formula:

$$V = \frac{H_d \sum_{i=1}^{8} \min(P_{di}, A_i) + H_n \sum_{i=1}^{8} \min(P_{ni}, A_i)}{24},$$

where H_d and H_n are the hours of daylight and darkness during the sampling period.

Total consumption (C_t) for each life history stage of walleye pollock was estimated by summing over the four predator groups (j):

$$C_t = \sum_{j=1}^{4} C_j \cdot S,$$

where S is stage duration (assumed to be 14 days for eggs and 7 days for yolk-sac larvae; Bailey and Stehr, 1986). The proportion of the total egg population consumed was estimated by dividing the number of eggs consumed by the number estimated to be available in the plankton at the nearest stations from the grid survey. This was not possible for yolk-sac larvae because of potentially high rates of extrusion through the 505-μm mesh nets (Lo, 1983).

Mesocosm study

Clearance rate experiments of predators feeding on walleye pollock eggs were conducted at sea during the 1988 and 1989 egg cruises. Polyethylene bags (300 l) were filled with filtered seawater, immersed in a large water bath at ambient temperature (3–4°C), and covered with a black tarp to simulate light at depth. Most (36) experiments were conducted in daytime for 9–12 hours, although a few (4) experiments were conducted for 20–24 hours. Four predator types (gammarids, hyperiids, euphausiids, and mysids) were removed live from Tucker trawls and held in seawater at 4–6°C. Eggs were extruded from trawl-caught adults, fertilized, and reared at 4–6°C. Eggs were counted into each bag, and 5–6 predators of one type were added. Initial egg densities were 0.01, 0.1, and 1 l^{-1} (low, medium, and high densities). Maximum egg densities estimated from oblique bongo tows in the Shelikof spawning area are from 0.2 to 0.6 l^{-1}, and actual densities over a narrower depth range would be considerably higher. From two to five replicate experiments were conducted for each density and predator type. Additional bags (one at low, two at medium, and three at high egg densities) without predators were used as controls for counting errors. Eggs were essentially neutrally buoyant. After each experiment, water from each bag was siphoned through a 333-μm mesh filter, the empty bag was rinsed three times, and the rinse water was likewise

filtered. Clearance rates ($l \cdot \text{predator}^{-1} \cdot \text{day}^{-1}$) were calculated as

$$C = \{(\ln(n_i) - \ln(n_f)) \cdot V\} \cdot (NT)^{-1},$$

where n_i is the initial egg density (number $\cdot$ l^{-1}), n_f is the final egg density (number $\cdot$ l^{-1}), V is the volume, N is the predator number, and T is the duration of the experiment in days (from Marin et al., 1986).

RESULTS

Seventeen likely zooplankton predator species were caught in Tucker trawls; mean densities ranged from 0.15 to 864.18 m^2 (Table 1). Except for euphausiids, the major categories were dominated by one species. Specimens frozen for gut analyses were not identified by species prior to immunoassays in 1988. However, on the basis of the sorted portion of catches, we assume that almost all gammarids were *Cyphocaris challengeri*; most hyperiids were *Themisto pacifica*; most mysids were *Meterythrops robusta*; and all euphausiids were *Euphausia pacifica*, *Thysanoessa spinifera*, and *T. inermis* in 1988.

Because there was little difference in vertical distribution among predator species within each major grouping, we have combined these distributions (Fig. 1). Walleye pollock eggs mainly (>80%) were distributed below 150 m. Most (>75%) small, presumably yolk-sac, pollock larvae (3–4 mm S.L.) were located above 150 m. There were marked diel changes in vertical distributions of all predators except mysids, which were caught near-bottom in both night and day tows. Gammarids were mostly near-bottom in daytime and more evenly distributed in midwater at night. Most euphausiids were caught above 150 m in day and night tows, with a shift toward the surface at night. Hyperiids were distributed nearly uniformly through the water column, especially at night.

From the egg cruises of both 1988 and 1989, 11–16% of gammarid and hyperiid amphipods tested positive for walleye pollock egg yolk proteins (Tables 2 and 3). Mysids in 1988 and 1989 and euphausiids in 1988

Table 1. Frequency of occurrence (F.O.) and mean ($\bar{x}$) and standard deviation (S.D.) of abundance by cruise per 10 m^2. Abundances include only individuals >5 mm in maximum dimension. (n = number of collections by cruise)

	Egg cruise, 1988			Yolk-sac cruise, 1988			Egg cruise, 1989		
Species	F.O. (n = 21)	($\bar{x}$)	S.D.	F.O. (n = 18)	($\bar{x}$)	S.D.	F.O. (n = 6)	($\bar{x}$)	S.D.
Gammaridea									
Cyphocaris challengeri	85.71	86.19	75.83	77.78	67.01	107.30	100.00	864.18	537.24
Atylus tridens	—	—	—	—	—	—	16.67	4.40	—
Hyperiidea									
Themisto pacifica	90.48	69.52	43.98	83.33	21.92	22.11	83.33	45.74	47.35
Themisto libella	4.76	0.15	—	—	—	—	—	—	—
Hyperia medusarum	4.76	1.01	—	—	—	—	—	—	—
Primno macropa	61.90	8.11	10.33	5.56	3.52	10.33	83.33	25.48	24.60
Mysidacea									
Meterythrops robusta	76.19	66.53	75.10	61.11	274.56	345.98	100.00	365.87	258.72
Holmesiella anomala	9.52	1.21	4.47	—	—	—	—	—	—
Archeomysis grebnitzkii	—	—	—	5.56	0.27	—	—	—	—
Xenocanthomysis spp.	—	—	—	5.56	22.19	—	—	—	—
Euphausiacea									
Euphausia pacifica	71.42	34.17	98.35	77.78	35.53	27.11	50.00	9.63	10.67
Thysanoessa spinifera	9.52	20.65	77.39	72.22	95.98	116.52	33.33	4.99	8.53
Thysanoessa inermis	23.81	20.85	59.02	72.22	113.89	139.88	66.67	121.10	241.23
Thysanoessa raschii	4.76	0.70	—	27.77	4.05	12.76	—	—	—
Thysanoessa inspinata	4.76	0.66	—	38.89	13.05	24.80	—	—	—
Thysanoessa longipes	28.57	6.30	11.73	22.22	3.72	9.80	33.33	16.03	11.41
Tessarabrachion oculatum	4.76	0.61	—	—	—	—	—	—	—

Figure 1. Vertical distribution of walleye pollock *Theragra chalcogramma* eggs and yolk-sac larvae and predator taxa from four MOCNESS tows (two day and two night) in the western Gulf of Alaska, April 21 and 24, 1988.

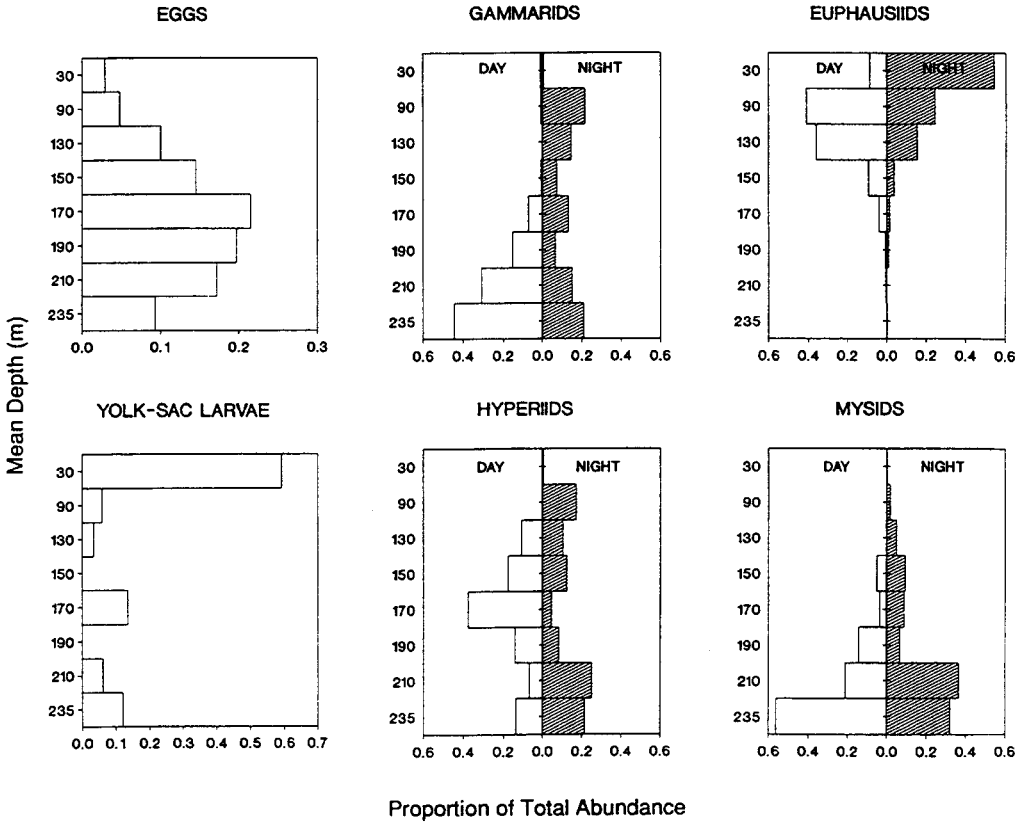


Table 2. Estimates of total number of eggs consumed by zooplankton predators during 1988 in relation to the number available per 10 m^2 of sea surface area.

Taxa	Mean abundance	Number analyzed	Positive reaction	Vertical overlap	Number consumed	Percent consumed
Gammaridea	86.19	103	0.144	0.577	602.5	5.39
Hyperiidea	69.52	114	0.113	0.708	467.3	4.18
Mysidacea	66.53	80	0.038	0.528	110.7	0.99
Euphausiacea	83.95	140	0.028	0.287	57.0	0.51
Total					1237.5	11.06

Table 3. Estimates of total number of eggs consumed by zooplankton predators during 1989 in relation to the number available per 10 m^2 of sea surface area.

Taxa	Mean abundance	Number analyzed	Positive reaction	Vertical overlap	Number consumed	Percent consumed
Gammaridea	867.14	55	0.164	0.577	6877.4	14.30
Hyperiidea	71.23	20	0.150	0.708	635.4	1.32
Mysidacea	365.87	93	0.054	0.528	872.4	1.81
Euphausiacea	141.15	2	0.028[1]	0.287	95.3	0.20
Total					8385.2	17.44

[1]Insufficient number analyzed for 1989, taken from 1988 analysis.

tested 3–5% positive. From the yolk-sac cruise in 1988, 15.5% of euphausiids tested positive for yolk proteins, and only 1.7% of gammarids had positive reactions (Table 4). We found no evidence of day/night differences in the incidence of pollock egg yolk proteins in guts of any of the four predator groups (chi-square test: all $P > 0.05$).

Detection time of yolk protein in euphausiid guts varied between 1 and 4 hours, and all detection of antigen ceased after 3–4 hours for hyperiid and gammarid amphipods. There was no discernable difference in digestion time between predators placed in filtered seawater and predators placed in seawater with algae. We have therefore made the simplifying, and conservative, assumption that detection time was equal to 4 hours for all predators (including mysids, which were not available to be tested).

In 1988, gammarid and hyperiid amphipods were the most significant consumers of pollock eggs (5.39 and 4.18%, respectively). In 1989, all predators except hyperiids were much more abundant, and these higher abundances, along with higher incidences of predation, resulted in an increase in the number of eggs consumed by all predator groups from 11.06% (0.8% day^{-1}) in 1988 to 17.44% (1.2% day^{-1}) in 1989 (Tables 2 and 3). Gammarid amphipods were again the most important consumers of eggs.

From the yolk-sac cruise in 1988, euphausiids were the most important consumers of yolk-sac larvae (Table 4), contrasting with the findings of the major predators on eggs in 1988 (Table 2).

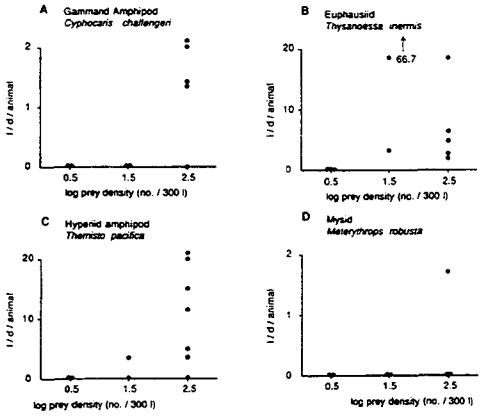
From bag experiments, clearance rates of the four predator types tested were greater at highest egg densities (i.e., at the three densities tested, the rates were not constant as expected) (Fig. 2). We suggest that this may have been because of the very low probability of a successful encounter at the lowest egg densities over the duration of the experiment. For example, at the highest egg densities the mean euphausiid clearance rate was only 0.3 l^{-1} $hour^{-1}$ $individual^{-1}$; therefore during a 12-hour experiment the probability of five euphausiids capturing an egg at the low density of 0.01 eggs l^{-1} is only about 16.6% (18 l cleared out of 300 l, with a probability of 0.01 that any one liter contains an egg: $1 - (99/100)^{18} = .1655$).

The number of eggs consumed in the sea, deter-

Table 4. Estimates of total number of yolk-sac larvae consumed by zooplankton predators during 1988 per 10 m^2 of sea surface area.

Taxa	Mean abundance	Number analyzed	Positive reaction	Vertical overlap	Number consumed
Gammaridea	67.01	59	0.017	0.322	15.1
Hyperiidea	25.47	51	0.078	0.331	27.8
Mysidacea	296.96	22	0.048	0.255	151.4
Euphausiacea	266.22	250	0.155	0.383	665.1
Total					859.4

Figure 2. Clearance rates of crustacean predators feeding on walleye pollock *Theragra chalcogramma* eggs in 300-l bags. The duration of experiments was 9–24 hours.



mined from mean and maximum clearance rates and observed densities of eggs and predators for 1988 and 1989, was compared with those from gut assays (Table 5). The number of eggs consumed from clearance rate experiments is 3–10 times lower than estimates derived from gut assays, except for euphausiids, which tend to have higher estimates based on clearance rates.

Immunoassays were conducted on guts of predators from the bag experiments. From 12 experiments in which no eggs were missing from the bags, 72 animals that presumably had eaten no eggs tested negative in immunoassays. From seven experiments in which eggs were consumed from the bags, four animals out of 42 tested positive.

DISCUSSION

Invertebrate predators are an important source of mortality for fish eggs and larvae (Hunter, 1981; Bailey and Houde, 1989), but estimates of their impact have been made only recently (Theilacker et al., 1986; Theilacker, 1988; Purcell, 1985, 1989; Purcell and Grover, 1990). In this study, we surveyed a range of abundant crustacean predators using an immunological assay and made initial estimates of predation potential in order to focus future efforts. Our results indicate that gammarid and hyperiid amphipods are important predators on walleye pollock eggs and that euphausiids are significant predators on yolk-sac larvae of this species.

Biological and physical factors can influence predation rates (Purcell, 1985; Bailey and Houde, 1989); among these factors is the spatial and temporal overlap in distribution of predators and prey (e.g., Frank and Leggett, 1982). We found that the vertical overlap of walleye pollock eggs and larvae with crustacean invertebrate predators had a major effect on estimates of predatory impact. Euphausiids, which occur mostly in the upper 150 m, overlapped minimally with eggs but overlapped substantially with yolk-sac larvae; euphausiids were the major predator group on yolk-sac larvae but ate relatively few eggs. Gammarid and hyperid amphipods, on the other hand, are most concentrated at middle or lower depths, overlap to a greater extent with the developing eggs, and apparently consume them at relatively high rates.

There are many factors that can influence estimates of predation from both laboratory experiments and field assay of gut contents. Predation rates from analysis of predator gut contents were generally much higher than estimates from laboratory-derived clearance rates.

Table 5. Comparison of total egg consumption estimated from clearance rate experiments and gut content immunoassays in 1988 and 1989 per 10 m^2 over the 14-day egg residence time. Range of egg consumption estimates are based on mean and maximum clearance rates for each predator taxon. Mean egg abundance used to calculate consumption from clearance rates was 11,000 per 10 m^2 in 1988 and 48,000 per 10 m^2 in 1989. Predator abundances and corrections for vertical overlap are the same as in Tables 2 and 3.

	Mean clearance rate	Maximum clearance rate	1988		1989	
Taxa	(l/day)	(l/day)	Estimated no. eaten	Estimated from guts	Estimated no. eaten	Estimated from guts
Gammaridea	0.8	2.0	6–15	602	282–675	6877
Hyperiidea	7.1	15.1	53–114	467	240–512	635
Mysidacea	0.6	1.8	3–10	110	78–233	872
Euphausiacea	7.1	18.9	26–70	57	192–515	95

There are several plausible reasons. First, capturing animals at depth with nets and bringing them to the surface could influence their physical condition and behavior, thus limiting their capabilities. Second, both *Cyphocaris* and *Themisto* tend to get trapped at the water–air interface in laboratory containers, effectively halting their feeding activity on suspended eggs. We did not notice this for individuals in the bags, but it remains a possible factor. Third, although the eggs appeared to be neutrally buoyant and well distributed in rearing jars, they may have been segregated from the predators in the bags, with predators accumulated at the sides, bottom, or top of the bags. This was probably a significant problem with mysids, which tend to remain near or on the bottom. From previous experiments conducted in 1-l jars, these same predators either did not eat fish eggs or ate them at low rates (Theilacker and Lasker, 1974; Bailey and Yen, 1983; Bailey and Stehr, 1986); however, they readily ate yolk-sac fish larvae. Confinement in small jars may also affect predator behavior, decreasing their ability to detect or handle fish eggs. De Lafontaine and Leggett (1987) found that for gelatinous predators, decreasing container size increased predation rates. Experiments by Monteleone and Duguay (1988) had different results; they found that clearance rates of the ctenophore *Mnemiopsis leiydi* decreased with decreasing container size. Predator species have different mechanisms for detecting and capturing prey; therefore their responses to container sizes are expected to vary.

Conversely, the immunoassays could have overestimated predation rates if there was codend feeding, if there were false positives, or if digestion rate estimates were too high. We used a codend with a mesh size that is considerably larger than the diameter of walleye pollock eggs, which should have minimized the capture of eggs and any codend feeding. Crustaceans used in bag experiments that did not consume eggs did not have any false positive reactions in the immunoassays. In addition, based upon animals that did consume eggs in bag experiments, the assays appeared to be relatively conservative. Our antibody-antigen reactions of predator gut contents was considerably weaker and more difficult to interpret than results of dilute yolk protein–antibody reactions. Antigenicity of the yolk protein was probably diminished during the digestion process. We suggest that in future studies, developing antibodies against partly digested (with trypsin or other enzyme) antigen be compared with antibodies against whole antigen. In addition, the reaction of predator guts with goat-anti-rabbit secondary antibody was a problem that we attempted to control by checking this reaction alone for each gut assayed. In retrospect, a nonreacting secondary antibody should be sought for future studies.

It was interesting that clearance rates estimated at low egg densities, similar to *in situ* values (0.01–0.1 eggs l^{-1}), were not constant, but rather increased with prey density. This observation could result from predators with low feeding rates combined with the short (10–24 hour) duration of experiments because the probability of ingesting prey is low. Experiments done with gelatinous zooplankton predators that feed upon fish eggs and larvae at higher rates and in which duration of experiments was longer (24–48 hours) have demonstrated constant clearance rates (Monteleone and Duguay, 1988; De Lafontaine and Leggett, 1987; Fancett and Jenkins, 1988); on the other hand, these are predators with linear or Type-I (Holling, 1959) functional responses. Animals that have a sigmoidal functional response, such as predators whose feeding activity decreases at low prey density, would be expected to have low clearance rates at low prey density.

Gut detection times of egg proteins were variable and not obviously influenced by alternative prey or continuous feeding. Immunoassay detection is based on the presence of antibody binding sites on the egg proteins. Digestion may influence the nature of these binding sites, and thus antigen digestion rates could be fast in relation to an assay based on total protein content. Thus our estimates of 4 hours for gut residence times is likely to be conservative. However, even halving detection times, thus doubling predation mortality, would not substantially alter our conclusions. Our antibody does not cross-react with algae or invertebrate eggs but does cross-react with yolk proteins from other fish species; in our field studies, cross-reactions with other fish eggs is an insignificant problem, since walleye pollock eggs comprised over 99% of all eggs caught in April cruises of 1988 and 1989 (Alaska Fisheries Science Center ichthyoplankton files, unpublished data).

Gut content assays may be conservative for several other reasons. First, predators may be defecating during the capture process. However, because the assay can detect nanogram amounts of egg protein, even a trace presence of yolk in the gut would be detected. Paradoxically, few predators from net catches were observed to have melanin in their guts compared with animals feeding in the laboratory. Although eggs have little melanin content until the eyes develop just before hatching, the absence of melanin may indicate, that defecation occurred during the capture process. Second, animals testing positive in the assays were assumed to have eaten only one prey during the digestion interval; this provides the most conservative esti-

mate and is supported by the low rate of feeding in the bag experiments. Finally, it is difficult to estimate the abundance of evasive predators such as euphausiids; problems with abundance estimates would apply equally to estimates of egg consumption from gut content and clearance rate methods.

Our estimates of daily egg mortality due to predation by pelagic crustaceans (0.8–1.2% day^{-1}) are low in relation to estimates of total egg mortality for walleye pollock (10–30% day^{-1} from Kim and Gunderson, 1989). The immunoassay results, as discussed above, may be conservative, but other sources of mortality could also be significant. For example, pelagic fishes may consume large numbers of eggs, or nonpredatory mortality (e.g., developmental abnormalities) could be substantial. Predation rates from gut assays were still considerably higher than those estimated from bag experiments, indicating that bag studies may underestimate the impact of predators. Because both the bag and immunoassay results depend on good estimates of predator abundance, we recommend improvements in this area. Our results also demonstrated the importance of vertical overlap of predators and prey, and this should be included as a component of future predation studies.

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